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Identification of a Fish Host of the Inflated Heelsplitter Potamilus inflatus (Bivalvia: Unionidae) with a Description of Its Glochidium

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ABSTRACT.—A survey of the fishes of the Black Warrior River was undertaken to determine fish host(s) of the federally threatened inflated heelsplitter, *Potamilus inflatus*. Seven hundred-twenty individual fishes representing 30 species were examined; mussel glochidia were found on 10 individual fishes representing nine species. *Potamilus inflatus* glochidia were only found infesting one freshwater drum (*Aplodinotus grunniens*), which is concordant with previous findings for the genus *Potamilus*. The morphology of *P. inflatus* glochidia is described and compared to *P. purpuratus*.

INTRODUCTION

Potamilus inflatus is a federally threatened mussel that inhabits large rivers in the southeastern United States (U.S. Fish and Wildlife Service, 1992). The historical range of *P. inflatus* has decreased markedly in the last decade prompting concern over the conservation status of this organism. Historically, the inflated heelsplitter was known from the Amite and Tangipahoa rivers in Louisiana, the Pearl and Tombigbee rivers in Mississippi, and the Black Warrior, Coosa and Tombigbee rivers in Alabama (Hurd, 1974; Stern, 1976; Hartfield, 1988). Presently it is limited to the lower and middle reaches of the Amite and Pearl rivers in Louisiana and in the Black Warrior River between the Demopolis Lock and Dam upstream to the Oliver Lock and Dam in Alabama (U.S. Fish and Wildlife Service, 1992). Little is known about the natural history of *P. inflatus*; however, such information is critical to effective conservation and species management.

Reproduction of mussels in the family Unionidae differs from other bivalves. Before fertilization the eggs pass into the suprabranchial chamber and then into the water tubes of the gills where they are fertilized (Pennak, 1989). The developing embryos are retained in the marsupium, a modified portion of the gill (Thorpe and Covich, 1991). Members of the genus *Potamilus* are long-term breeders; the eggs are fertilized in the summer and the embryos are not released for almost a year (Heard and Guckert, 1970). A critical stage in the development of all unionid mussels is the attachment of the glochidium larvae on a suitable fish host. After attachment to a host, the glochidium is encysted as the tissue of the fish grows to cover it (Pennak, 1989). During this stage the juvenile mussels of some

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species develop their adult shell and anatomy (Surber, 1912, 1913, 1915; Cummings *et al.*, 1990) that will enable them to begin life as a filter-feeding member of the benthic community. While all species of unionids do not appear to be host-specific, the genus *Potamilus* parasitizes the freshwater drum (*Aplodinotus grunniens*) almost exclusively (Surber, 1913; Wilson, 1916; Cummings *et al.*, 1990). A single exception was reported by Surber (1913) who found glochidia of *P. ohiensis* on white crappie (*Pomoxis annularis*).

The objective of this study was to identify the fish host(s) of *Potamilus inflatus*. Such information may prove useful in management and recovery of the species, as unionids are dependent upon their fish hosts during a critical period of their natural history. Two species of *Potamilus* (*P. inflatus* and *P. purpuratus*) are present in the Black Warrior River. All described glochidia of *Potamilus* can be distinguished from those of other unionids by their axe-head shape (Hoggarth, 1988). However, the glochidium of *P. inflatus* have not been previously described. In order to facilitate the identification of these taxa, we describe the glochidium of *P. inflatus* and compare it to the glochidium of *P. purpuratus*.

METHODS

Ten collections of fishes were made twice a week between 27 June and 28 July 1995 in the Black Warrior river at 12 Mile Rock, (river mile 327.3), Tuscaloosa Co., Alabama. A single collection was made at Choctaw Bend, Greene Co., Alabama, below the Warrior Dam (between river mile 262 and 261) on 14 July 1995. These sites were selected because those portions of the river were known to contain populations of *Potamilus inflatus* (Williams *et al.*, 1992). Several collection methods were employed including gill netting, seining and hook and line. Fishes were preserved in a solution of 10% formalin and examined for glochidia on the fins and gills with a dissecting microscope. Gills harboring glochidia were isolated and placed in a solution of 70% ethanol for later identification using a compound microscope. Glochidia were identified based on their morphology as described in the following section. Common and scientific names of fishes follow Robins *et al.* (1991). Previous surveys by one of us (PH) indicated that glochidia release occurred between June and July. A preliminary survey on 27 June 1995 of adult *P. inflatus* at 12 Mile Rock indicated that female *P. inflatus* were releasing glochidia. This was determined by opening the valves slightly by hand and visually inspecting the marsupium. Several of the female mussels examined had partially discharged water tubes, indicating they were in the process of releasing glochidia. To increase the likelihood of collecting fish infested with *P. inflatus* glochidia, we concentrated our efforts in this area. No attempt was made to identify the other glochidia found during this study, other than to confirm they were not *P. inflatus*.

A single female *Potamilus inflatus* was collected during the preliminary survey on 27 June and maintained in an aquarium with river water and sediment until it had released its glochidia. Glochidia were recovered from the substrate with an eye-dropper. *Potamilus purpuratus* glochidia were obtained from a preserved female specimen collected on 30 June 1993 from the Cahaba River, Bibb Co., Alabama. Measurements of 10 glochidia of each species were made with an ocular micrometer. Glochidia height is defined as the greatest distance between the dorsal and ventral margins; dorsal length is defined as the greatest length between the anterior and posterior edges along the dorsal margin, and ventral length is defined as the greatest distance between the anterior and posterior edges along the ventral margin. Glochidia were prepared for scanning electron microscopy (SEM) following procedures outlined in Hoggarth (1988). The features identified using SEM were subsequently used to identify glochidia encysted in gill tissues. Gill tissue containing glochidia was prepared by partial clearing in a solution of 10% trypsin and sodium borate. The prepared tissue was examined and the glochidia identified using a compound microscope.

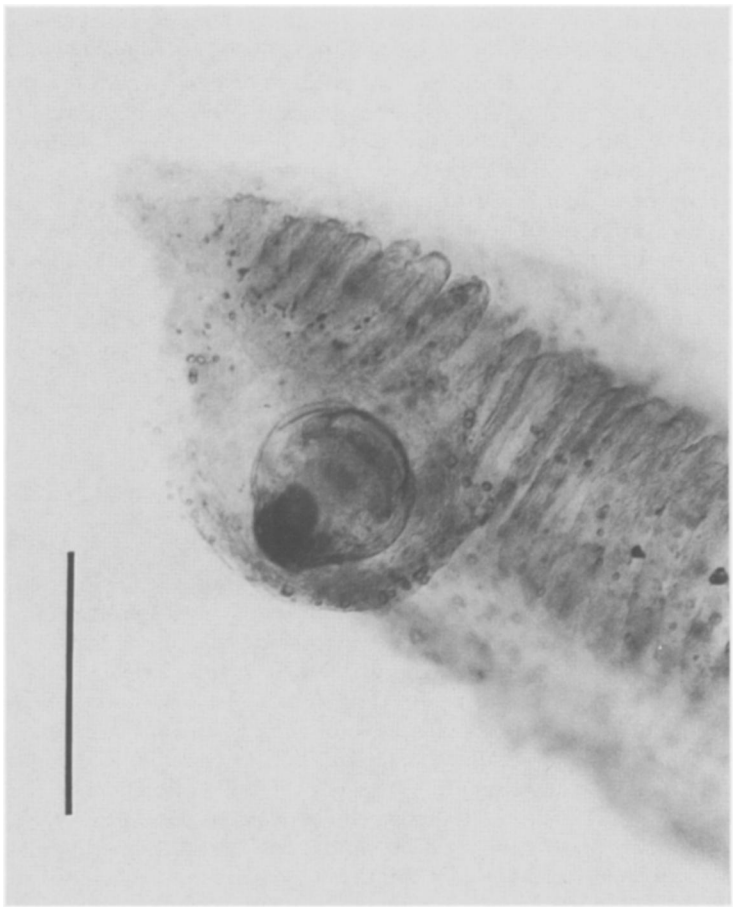

FIG. 1.—Light micrograph of *Potamilus inflatus* larvae encysted in gill filament of *Aplodinotus grunniens*. Bar = 0.20 mm

RESULTS

A total of 720 fishes representing 30 species were collected and examined for glochidial infestation (Table 1). The number of fish species examined represented 61% of those reported by Mettee *et al.* (1989) between the Oliver Lock and Dam and the Warrior Lock and Dam. Ten of the 720 specimens were infested with glochidia and one of the 37 specimens of *Aplodinotus grunniens* (collected 10 July 1995) was infested with 12 glochidia identified as *Potamilus inflatus*. All glochidia were attached to or encysted in the gills; no glochidia were observed on the fins of any fishes examined (Fig. 1).

Description of glochidia.—The glochidia of *Potamilus purpuratus* and *P. inflatus* were readily distinguishable from each other. The glochidia of *P. inflatus* are small, with a mean height of 0.188 mm (SD = 0.01 mm, range = 0.180–0.234 mm) and axe-head shaped (Fig. 2a,b). The dorsal margin is straight, with a mean length of 0.070 mm (SD = 0.008 mm, range = 0.054–0.081) and the ventral margin is curved with a mean length of 0.125 mm (SD = 0.020, range = 0.081–0.126 mm) Large lanceolate hooks are present on the anterior and posterior edges of

TABLE 1.—List of fishes[1] reported from the Black Warrier River between the William Bacon Oliver Lock and Dam and the Armisted I. Selden Lock and Dam (Mettee *et al.*, 1989) with numbers of each species examined for this survey

Species	N	Glochidia	Species	N	Glochidia
Lepisosteus oculatus	8	−	*Notropis atherinoides*	13	−
L. osseus	—		*N. candidus*	75	−
Amia calva	—		*N. edwardraneyi*	213	−
Alosa chrysochloris	17	−	*N. texanus*	1	−
Dorosoma cepedianum	49	−	*Opsopoeodus emiliae*	—	
D. petenense	59	−	*Pimephales vigilax*	47	−
Esox niger	—		*Carpiodes cyrpinus*	5	−
Cyprinella venusta	29	−	*C. velifer*	11	+
**Cyprinus carpio*	1	−	*Ictiobus bubalus*	2	−
Hybopsis winchelli	—		*Moxostoma erythrurum*	—	
Macrhybopsis storeriana	9	−	*M. poecilurum*	—	
Ictalurus furcatus	2	−	*Amieurus natalis*	—	
I. punctatus	17	+	*Lepomis macrochirus*	22	+
Pylodictus olivaris	—		*L. megalotis*	36	+
**Aphrododerus sayanus*	1	−	*L. microlophus*	21	+
Strongylura marina	5	−	*L. punctatus*	—	
Fundulus olivaceus	—		*Micropterus punctulatus*	20	+
Gambusia affinis	1	−	*M. salmoides*	1	−
Labidesthes sicculus	2	−	*Pomoxis annularis*	1	−
Morone chrysops	—		*P. nigromaculatus*	—	
**M. mississippiensis*	14	+	*Percina shumardi*	—	
M. chrysops × *saxatilis*	1	−	*P. vigil*	—	
			Aplodinotus grunniens	37	+

[1] List includes fishes caught at Choctaw Bend on 14 July 1995: *Cyprinella venusta* (5), *Notropis atherinoides* (3), *N. edwardraneyi* (5), *Ictalurus punctatus* (1), *Labidesthes sicculus* (1), *Lepomis macrochirus* (1), *L. megalotis* (9), *L. microlophus* (4), *Micropterus punctulatus* (5), *M. salmoides* (1)

* Indicates fishes not reported by Mettee *et al.*, 1989

both valves. These hooks extend more antero-posteriorly in *P. inflatus* than in other congeners (Hoggarth, 1988). Between these large hooks are a variable number (5–7) of smaller bifurcate hooks. Micropoints are present on the ventral edge of both valves, with some extending onto the base of the smaller hooks. The micropoints are lanceolate and are loosely organized into vertical rows and the valves are equal in size with no lateral valve gape.

The glochidia of *Potamilus purpuratus* are twice as large as *P. inflatus*, averaging 0.371 mm in height (SD = 0.001 mm, range = 0.360–0.378 mm) and are elongate and strap-like (Fig. 2c,d). The dorsal margin is straight, mean length = 0.108 mm (SD = 0.015, range 0.072–0.126 mm) while the ventral margin is only slightly curved, mean length = 0.201 mm (SD = 0.015, range = 0.180–0.220). *Potamilus purpuratus* glochidia possess large lanceolate hooks on the anterior and posterior margins of the valves, oriented at nearly a right angle to the antero-posterior plane and lack the smaller bifurcate hooks found on *P. inflatus*. Micropoints are also present on the ventral margins of both valves and are organized into vertical rows. The valves are unequal ventrally with one side fitting within the other. A large lateral valve gape is present.

The *Potamilus inflatus* glochidia observed on the drum were well-encysted in the gill tissue of their host. All glochidia were located at or near the distal end of the gill filament.

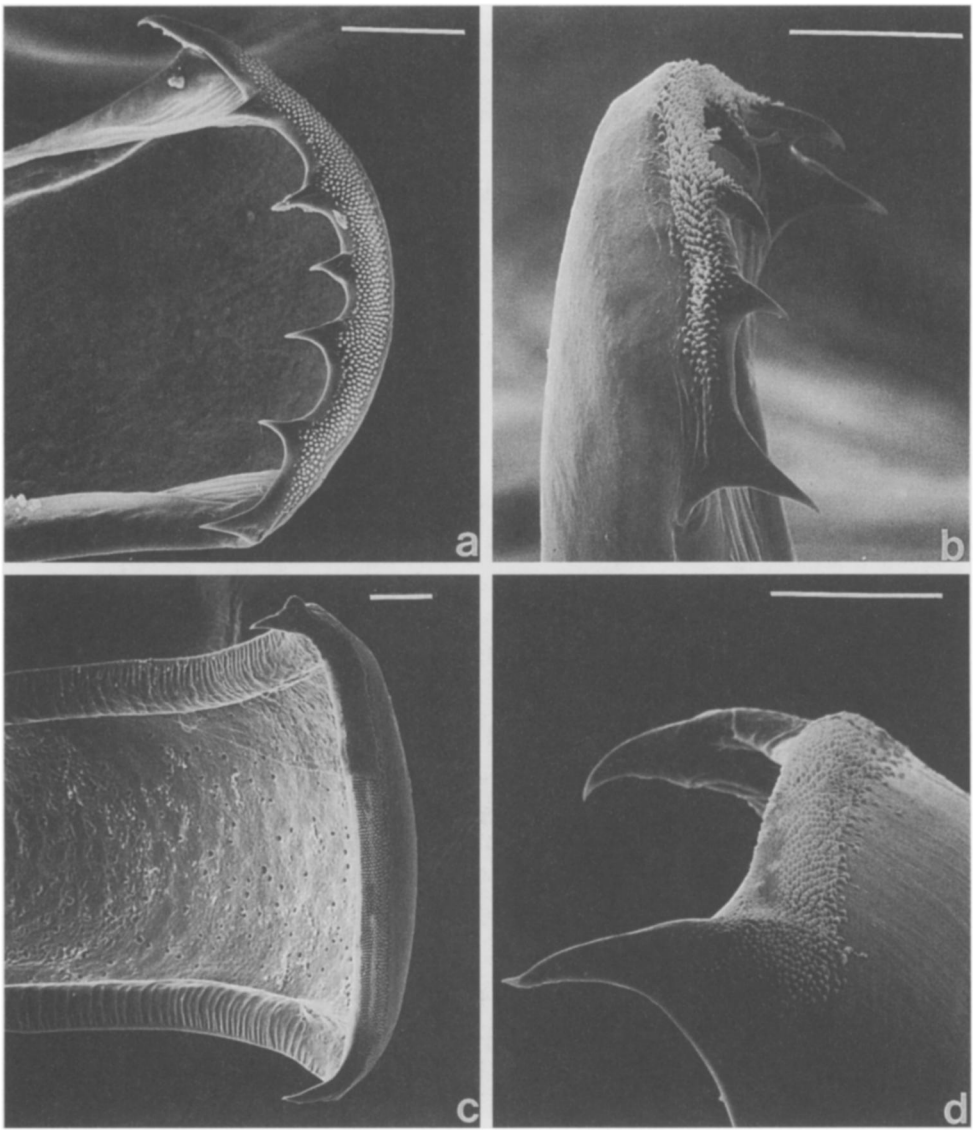

FIG. 2.—Scanning electron micrographs of glochidia larvae. 2a,b medial and lateral views larvae of *Potamilus inflatus*. 2c,d medial and lateral views larvae of *Potamilus purpuratus*. Bar = 25 μm

The axe-head shape, large lanceolate hooks, and smaller hooks were present confirming that these were *P. inflatus* glochidia. In addition, the adult valves were clearly visible, extending beyond the margins of the glochidial valves (Fig. 1).

DISCUSSION

The presence of metamorphosing *Potamilus inflatus* glochidia encysted on a freshwater drum indicates that drum are a host for *P. inflatus*. Although female *P. inflatus* were actively

discharging glochidia when the study began, only one of 37 freshwater drum collected (2.7%) was infected with their glochidia. Low infection rates might be due to: (1) low numbers of host fish encountering gravid female *P. inflatus*; (2) low numbers of gravid female mussels or (3) the reflection of normal infestation rates for this species. Freshwater drum are widespread and relatively common in the Black Warrior River (Mettee *et al.*, 1989); however, freshwater drum may have habitat preferences that preclude their encountering *P. inflatus* and are therefore not locally abundant in stretches of the river inhabited by mussels. Swingle (1953) found a significant decrease in the abundance of freshwater drum following river impoundments, and hypothesized that flowing water was necessary for reproduction of the fish. The Black Warrior River is impounded above and below the section of the river where the gravid *P. inflatus* were found, and this stretch exhibited little flow. Changes in the river flow due to impoundment may explain the low infection rates observed: drum do not frequent areas with low flow and are therefore unlikely to encounter the mussels and subsequently become infected.

Mean densities of *Potamilus inflatus* in the Black Warrior River are low, although at some sites surveyed they were the dominant species of mussel (Miller *et al.*, 1996). Values ranged from 0.5 individuals/100 m^2 to 0.97 individuals/100 m^2 depending on which substrate they were found (Miller *et al.*, 1996). These densities might explain the low infestation rates found in this study. However, previously reported infestation rates for other species of *Potamilus* are comparable to those found in this study. Surber (1913) indicated that only 2% of the *Aplodinotus grunniens* in his survey were infested with the glochidia of *P. ohiensis*. Weiss and Layzer (1995) studied glochidial infestations of the fishes of the Barren River, Kentucky, and found that 3% (n = 73) of drum were infested with glochidia of *P. alatus*. In contrast, Cummings *et al.* (1990) documented a comparatively high infestation rate for *P. capax*, where 75% of the *A. grunniens* collected were infected with glochidia of *P. capax*. The low infestation rate of drum by *Potamilus glochidia* observed in this and other studies may be typical for this genus. Although Cummings *et al.* (1990) observed a much higher rate than we observed, we note that their sample size was small (n = 8) possibly biasing the observed infestation rate. Further investigations into the reproductive biology of this species are needed to determine what other factors may be influential in the low infestation rates observed in this and other studies.

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